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Exhibit A

Pharmacology and Biological Efficacy of a Recombinant, Humanized, Single-Chain Antibody C5 Complement Inhibitor in Patients Undergoing Coronary Artery Bypass Graft Surgery With Cardiopulmonary Bypass

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Background—Cardiopulmonary bypass (CPB) induces a systemic inflammatory response that causes substantial clinical morbidity. Activation of complement during CPB contributes significantly to this inflammatory process. We examined the capability of a novel therapeutic complement inhibitor to prevent pathological complement activation and tissue injury in patients undergoing CPB.

Methods and Results—A humanized, recombinant, single-chain antibody specific for human C5, h5G1.1-scFv, was intravenously administered in 1 of 4 doses ranging from 0.2 to 2.0 mg/kg before CPB. h5G1.1-scFv was found to be safe and well tolerated. Pharmacokinetic analysis revealed a sustained half-life from 7.0 to 14.5 hours. Pharmacodynamic analysis demonstrated significant dose-dependent inhibition of complement hemolytic activity for up to 14 hours at 2 mg/kg. The generation of proinflammatory complement byproducts (sC5b-9) was effectively inhibited in a dose-dependent fashion. Leukocyte activation, as measured by surface expression of CD11b, was reduced ($P<0.05$) in patients who received 1 and 2 mg/kg. There was a 40% reduction in myocardial injury (creatine kinase–MB release, $P=0.05$) in patients who received 2 mg/kg. Sequential Mini-Mental State Examinations (MMSE) demonstrated an 80% reduction in new cognitive deficits ($P<0.05$) in patients treated with 2 mg/kg. Finally, there was a 1-U reduction in postoperative blood loss ($P<0.05$) in patients who received 1 or 2 mg/kg.

Conclusions—A single-chain antibody specific for human C5 is a safe and effective inhibitor of pathological complement activation in patients undergoing CPB. In addition to significantly reducing sC5b-9 formation and leukocyte CD11b expression, C5 inhibition significantly attenuates postoperative myocardial injury, cognitive deficits, and blood loss. These data suggest that C5 inhibition may represent a novel therapeutic strategy for preventing complement-mediated inflammation and tissue injury. (***Circulation.*** 1999;100:2499-2506.)

Key Words: cardiopulmonary bypass ■ inflammation ■ proteins ■ immunology

Cardiopulmonary bypass (CPB) elicits a systemic inflammatory response that causes tissue injury and contributes to significant perioperative and long-term clinical morbidity.[1–4] During CPB, exposure of blood to bioincompatible surfaces of the extracorporeal circuit, as well as tissue ischemia and reperfusion associated with the procedure, induces the activation of several major humoral pathways of inflammation.[1–4] These various pathways include the complement, coagulation, and cytokine cascades. The products of these proinflammatory pathways promote the recruitment and activation of inflammatory leukocytes, resulting in the elaboration of inflammatory mediators, with subsequent tissue injury and functional impairment.[5–7]

Clinical manifestations attributed to this systemic inflammatory response involve morbidity to multiple organ systems, including the heart, brain, blood, lung, kidney, and gastrointestinal tract.[1,4,8] Myocardial injury may manifest as perioperative Q-wave or non–Q-wave myocardial infarction (MI) or

as severe ventricular dysfunction requiring circulatory assist.[1] Systemic inflammation, together with diffuse cerebral micro-embolization,[9] may result in clinically significant stroke or, more commonly, in cognitive deficits that persist in a significant proportion of patients.[9,10] Additional clinical sequelae of CPB-induced inflammation and ischemia include impaired hemostasis, pulmonary edema, renal insufficiency, and gastrointestinal dysfunction.[4,11]

Among the inflammatory cascades, activated components of the complement system contribute to all phases of the inflammatory response.[12] In particular, the products that are generated after cleavage of C5, namely, C5a and C5b-9, are potent inflammatory mediators with pleiotropic activities that include alteration of blood vessel permeability and tone, leukocyte chemotaxis, and activation of multiple inflammatory cell types.[12] The generation of these byproducts during CPB is well documented and has been shown to correlate with clinical morbidity.[13–15] Inhibition of complement activation at C5 would prevent the formation of these proinflammatory molecules while allowing the generation of upstream products, such as C3b, the critical mediator of bacterial opsonization as well as immune complex solubilization and clearance. C5 inhibition therefore represents a potentially effective therapeutic modality for reducing CPB-induced inflammation.

Using a novel approach to complement inhibition, we have developed a 25-kDa recombinant, humanized, single-chain antibody (h5G1.1-scFv) that binds to human C5 with very high affinity ($\approx$100 pmol/L) and thereby blocks C5 cleavage by both the classic and alternative complement pathway C5 convertases.[16] In preclinical studies, antibody-mediated C5 inhibition markedly reduced inflammation and tissue damage in models of bypass-associated bioincompatibility,[17] myocardial ischemia, and reperfusion.[18] This investigation reports the first clinical studies examining the safety, pharmacology, and initial biological and clinical efficacy of the h5G1.1-scFv C5 inhibitor in patients undergoing CABG surgery with CPB.

Methods

Study Design

This investigation was designed as a prospective, open-label, randomized, dose-escalation study conducted at Yale–New Haven Hospital (New Haven, Conn) and Brigham and Women's Hospital (Boston, Mass), between December 1996 and October 1997. After Human Investigation Committee approval and informed consent were obtained, 35 patients undergoing primary, nonemergent CABG surgery with CPB were enrolled in the study. Patients with an active infection, complement deficiency, prior exposure to monoclonal antibodies (mAbs), ejection fraction <30%, malignancy, corticosteroid use within the previous 7 days, or a history of hematologic, hepatic, or renal disease were excluded.

The study was performed in 2 phases. In phase 1, 17 patients were randomly assigned to 1 of 4 groups (n=4) receiving 0.2, 0.5, 1.0, or 2.0 mg/kg of h5G1.1-scFv. In each group, 1 patient randomly received placebo. In phase 2, 18 patients were randomly assigned to 3 groups (n=6) receiving placebo or 1.0 or 2.0 mg/kg h5G1.1-scFv.

Study Procedures

All potential patients were screened before enrollment. Once all inclusion and exclusion criteria were satisfied, patients were randomized. Perioperative testing included medical history and physical examination, laboratory testing (hematology, chemistry, and urinal-

ysis), ECG, and Mini-Mental State Examination (MMSE). Data collection included chest-tube output and transfusion requirements.

Systemic heparin was administered before CPB. Once the activated clotting time was greater than 400 seconds, h5G1.1-scFv was infused over a 10-minute period, followed by initiation of CPB. Although the conduct of anesthesia and surgery was similar for each institution, no attempts were made to standardize techniques. CPB was conducted with hemodilution, moderate hypothermia, and membrane oxygenators.

After surgery, patients were transported to the intensive care unit for 24 to 48 hours and then transferred to a telemetry unit until hospital discharge. Patients were seen 4 to 6 weeks after surgery for a termination interview and blood sampling.

Blood-Sample Collection

Blood samples were drawn before, during, and after CPB at predetermined intervals: before heparinization, 5 minutes after administration of h5G1.1-scFv, after 5 minutes at 28°C, after initiation of rewarming, after 5 minutes at 37°C, after CPB (5 minutes and 2, 7, 12, 18, 24, 36, and 48 hours after CPB), at hospital discharge, and 4 to 6 weeks after surgery. All samples were immediately centrifuged at 4°C and stored at −70°C until they were assayed.

h5G1.1-scFv Study Drug

The study drug h5G1.1-scFv (Alexion Pharmaceuticals, Inc, New Haven, Conn) is a recombinant, fully humanized, single-chain antibody with picomolar affinity for a sequence within human C5.[16] It was supplied as a sterile, nonpyrogenic solution (2 mg/mL) for intravenous injection.

Monoclonal Antibodies

h5G1.1-scFv was produced as a recombinant protein in *Escherichia coli* and purified under GMP conditions as described previously.[16] Anti-CD45 (2D1, Becton-Dickinson) and anti-CD11b (P6.2, Exalpha) were used for leukocyte labeling.

Biological Assays

Pharmacokinetics of h5G1.1-scFv were determined by a double sandwich ELISA (NUNC). This assay detects both free and C5-bound h5G1.1-scFv and thus reflects the total h5G1.1-scFv in the serum. Briefly, ELISA plates were coated with rabbit anti-mouse IgG1 antibody (Zymed) and then blocked with PBS containing Tween 20 (Sigma). After they were washed, the plates were incubated with mouse anti–h5G1.1-scFv (6A8). Serum samples were added and incubated. After the final washing was performed, anti-mouse IgG2b (7H7) antibody was added, and the plates were developed with peroxidase substrate (Zymed). Total serum complement hemolytic assays (pharmacodynamics) were performed as described previously.[17] For measurement of fluid-phase C3a (C3a des-arg) and sC5b-9, serum was diluted in sample preservative solution (Quidel) immediately before freezing, then determinations were made as described previously.[17]

Creatine Kinase–MB Assay

Myocardial-specific isoforms of creatine kinase (CK) were measured in the clinical laboratories of the 2 hospitals by a standard technique.

Fluorescence Labeling of Leukocytes and Flow Cytometry

Whole-blood samples were immediately fixed in paraformaldehyde PBS for flow cytometry studies.[17] Samples were prepared for labeling, incubated with saturating concentrations of mAb, and then prepared for fluorescence-activated cell sorter (FACS) analysis as previously detailed.[17] Samples were analyzed on a FACScan flow cytometer (Becton-Dickinson). For determination of leukocyte activation, samples were labeled with FITC-anti-CD45 or PE-anti-CD11b. Leukocyte measurements were performed by live gating on FITC-positive, leukocyte-sized events with forward- versus side-

scatter parameters used to differentiate between monocyte and neutrophil subsets.

Cognitive Function Testing

The MMSE[19] was administered by a single individual at each site to each patient at screening, on postoperative day 1, and at hospital discharge. The MMSE was used to quantify global cognitive function. However, the measured degree of cognitive decline in this and other studies is influenced not only by intervention but also by the sensitivity and specificity of the tests used.[20]

Statistical Analysis

All data are presented as mean±SEM. Statistical analysis (True Epistat version 5.1, EpiStat and InStat version 3.01, GraphPad) was performed by multivariate ANOVA for repeated measurements over time and a 1-way ANOVA with Tukey's multiple comparison procedure for continuous variables. Categorical variables, including cognitive deficits, were analyzed by χ^2 analysis or Fisher's exact tests. Statistical significance was defined as a P value <0.05.

Results

Demographics and Safety

Demographic variables did not significantly differ among patient groups (data not shown). Review of hematologic and chemistry laboratory values showed no substantial differences among groups (data not shown). In addition, adverse events were reported in 10 of 10 placebo and 24 of 25 h5G1.1-scFv patients, with the most commonly reported adverse events including fever, dysrhythmias, and atelectasis. Serious adverse events were reported in 1 of 10 placebo and 4 of 25 h5G1.1-scFv patients and included pericarditis, protamine reaction, sternal wound infection, perioperative MI, and ventricular tachycardia. The adverse events and serious adverse events were not significantly different among groups. Therefore, h5G1.1-scFv was clinically safe and well tolerated in patients undergoing CPB.

Pharmacokinetics and Biodistribution

Previous studies on the pharmacokinetics of single-chain antibodies have demonstrated rapid clearance from the serum, with serum half-lives ($t_{1/2}$) ranging from 15 to 30 minutes.[21] The pharmacokinetic profile of h5G1.1-scFv was determined from serum levels after a single bolus administration before CPB. A dose-dependent increase in serum levels of h5G1.1-scFv persisted throughout the 26-hour monitoring period (Figure 1A), and a biphasic decline was noted at all doses. h5G1.1-scFv was not detected in any serum sample collected 5 to 7 days after drug administration (data not shown). The initial deposition phase for all doses occurred within 4 hours of administration and was followed by a prolonged terminal elimination phase. The $t_{1/2}$ was decreased at lower doses of h5G1.1-scFv (7.0 to 8.9 hours) compared with the 2.0 mg/kg dose (14.5 hours). This substantial prolongation in the $t_{1/2}$ of h5G1.1-scFv relative to other single-chain antibodies is most likely due to its high affinity binding to ($K_d = 100$ pmol/L) and slow dissociation rate from ($K_{off} = 1.0 \times 10^{-4}$) the 190-kDa human C5 protein.[16]

Biodistribution of h5G1.1-scFv was determined for the 1.0 and 2.0 mg/kg doses (Figure 1B) 4 hours after administration. There was no significant difference in the renal clearance of h5G1.1-scFv at either dose. There was, however, a dose-
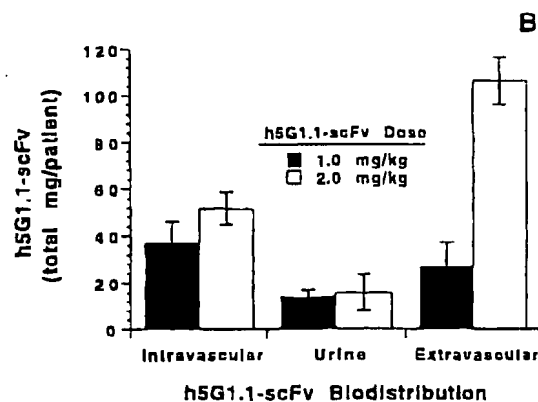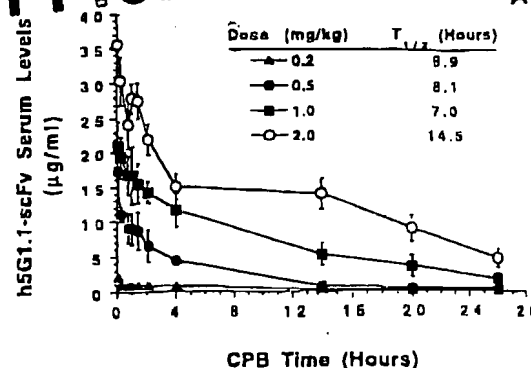

Figure 1. Pharmacokinetics and biodistribution of h5G1.1-scFv in CPB patients. h5G1.1-scFv was administered to CPB patients as a single bolus (0, 0.2, 0.5, 1.0, and 2.0 mg/kg) over 10 minutes just before CPB. A, Pharmacokinetics. Serum levels of h5G1.1-scFv were determined at various time points during and after CPB by a standard double sandwich ELISA. Data shown represent mean calculated $t_{1/2}$ for each dose±SEM for each time point. B, Biodistribution. Serum (intravascular) and urine levels of h5G1.1-scFv were determined at 4 hours after h5G1.1-scFv administration by a standard double sandwich ELISA. Extravascular h5G1.1-scFv component was determined by subtraction of intravascular and urine levels from original bolus dose. Data shown represent mean biodistribution of h5G1.1-scFv at 4 hours after administration±SEM.

dependent increase in the intravascular distribution (37.1 versus 51.5 mg) and a 4-fold increase in the extravascular distribution (26.8 versus 106 mg) of h5G1.1-scFv. The pharmacokinetic profile of the 2.0 mg/kg dose suggests that the prolonged $t_{1/2}$ of h5G1.1-scFv (14.5 hours) resulted from a return of the extravascular pool of drug into the intravascular space during the 26-hour monitoring period.

Pharmacodynamics

Serum complement hemolytic activity was measured to assess the efficacy of h5G1.1-scFv–mediated complement inhibition. Our previous studies[16] have shown that h5G1.1-scFv binds to C5 in serum, resulting in a dose-dependent inhibition of total serum hemolytic activity. Serum complement hemolytic activity (pharmacodynamics) was not significantly inhibited in patients who
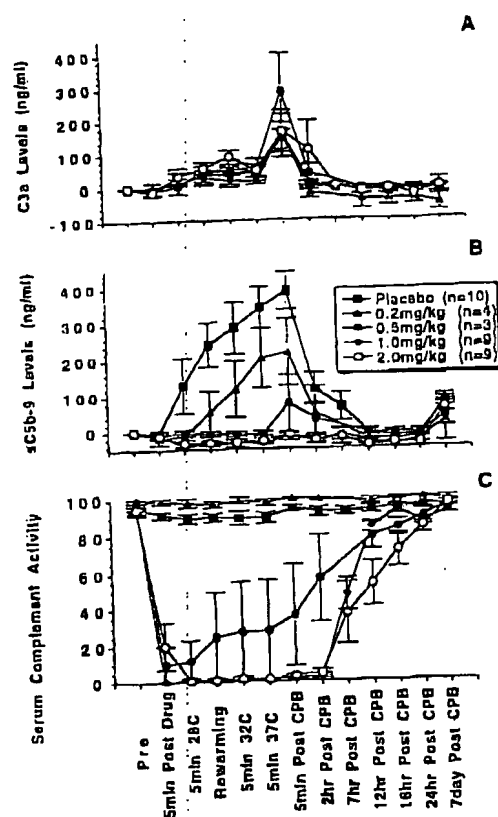
Figure 2. Complement activation and serum complement activity in CPB patients treated with h5G1.1-scFv. Levels of C3a (A), sC5b-9 (B), and serum complement activity (C) were measured in serum samples drawn from CPB patients. Complement activation (C3a and sC5b-9 levels) was determined by ELISA, and serum complement activity was determined by hemolytic assay. Data shown represent mean±SEM for all data points.

received 0.2 mg/kg h5G1.1-scFv. However, hemolytic activity was significantly ($P<0.05$) inhibited in a dose-dependent fashion by all remaining doses of h5G1.1-scFv compared with placebo (Figure 2C). Hemolytic activity dropped markedly 5 minutes after administration of 0.5 mg/kg h5G1.1-scFv and gradually returned to normal over a 12-hour period. Administration of h5G1.1-scFv at the 1.0 and 2.0 mg/kg doses completely inhibited hemolytic activity for >2 hours after CPB. In addition, the 2.0 mg/kg dose substantially reduced (>50%) hemolytic activity for >14 hours. The pharmacodynamic profile of the 2.0 mg/kg dose was consistent with the pharmacokinetic analysis, which demonstrated a substantial difference in serum levels 12 hours after CPB (Figure 1A).

Analysis of Complement Activation

Serum C3a and sC5b-9 levels were measured to demonstrate the effect of h5G1.1-scFv on the generation of activated complement components. C3a levels did not significantly differ between patients who received placebo and those who received h5G1.1-scFv (Figure 2A). In contrast, sC5b-9 levels were significantly ($P<0.05$) decreased in a dose-dependent manner in patients treated with h5G1.1-scFv (50%, 90%, >99%, and >99% for the 0.2, 0.5, 1.0, and 2.0 mg/kg doses, respectively) compared with placebo (Figure 2B).
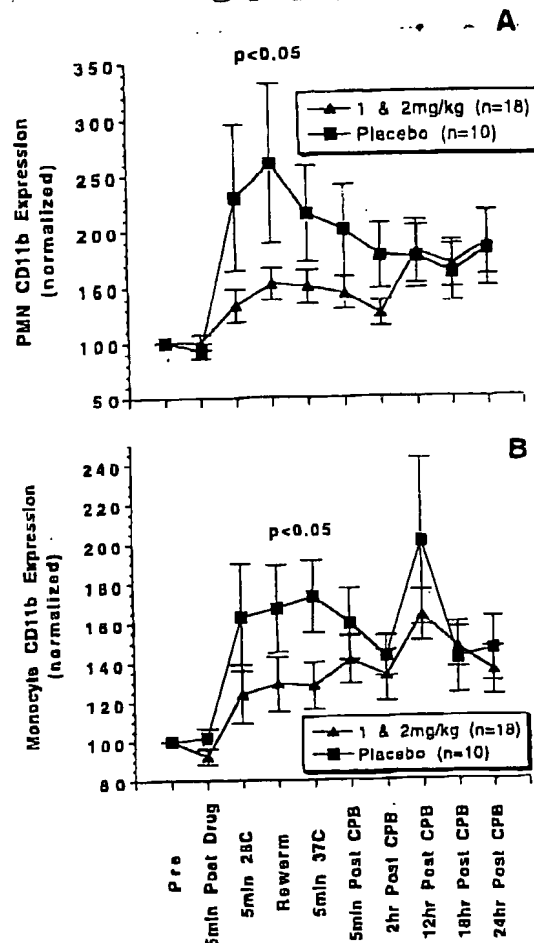

Figure 3. Analysis of leukocyte CD11b expression in CPB patients treated with h5G1.1-scFv. CD11b expression was measured on peripheral blood leukocytes (polymorphonuclear leukocytes [PMN] in A and monocytes in B) after single-bolus administration of h5G1.1-scFv (0.0 vs 1.0 and 2.0 mg/kg). CD11b was measured by fluorescence labeling (FACS analysis) and is expressed as percentage of baseline values. Data shown represent mean±SEM for all time points.

Analysis of Leukocyte Activation

CD11b expression was measured on activated neutrophils and monocytes (Figure 3A and 3B). In doses sufficient to completely block hemolytic activity and sC5b-9 generation (1.0 and 2.0 mg/kg), h5G1.1-scFv significantly ($P<0.05$) attenuated peak leukocyte CD11b expression compared with placebo.

Analysis of Myocardial Injury

Soluble C5b-9 production[22] correlates with myocardial injury during CPB.[23-25] To assess myocardial injury, the total release of CK-MB was measured during the 24 hours after drug administration. Total CK-MB was significantly less ($P<0.05$) in patients treated with 2.0 mg/kg h5G1.1-scFv than in those given placebo (704±166 versus 1245±449 IU/mL; Figure 4).

Cognitive Deficits

Preoperative and postoperative cognitive performance were assessed with the MMSE (Figure 5). Compared with
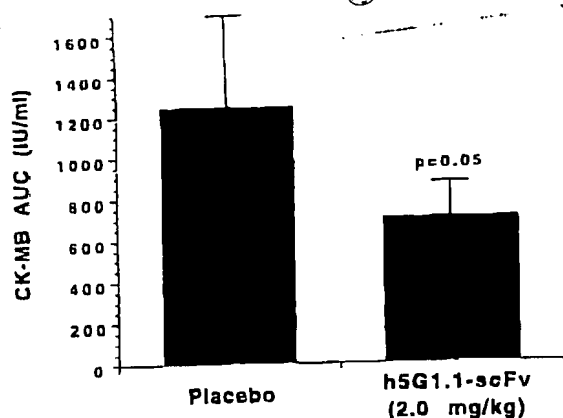
Figure 4. Analysis of myocardial injury in CPB patients treated with h5G1.1-scFv. Myocardial injury was determined in CPB patients by measurement of the cumulative release of CK-MB over 24 hours. Data shown represent mean±SEM for CK-MB measurements over 24 hours for patients who received placebo (n=10) vs 2.0 mg/kg h5G1.1-scFv (n=9). AUC indicates area under the curve.

preoperative scores for all patient groups, cognitive performance on the MMSE was significantly worse on postoperative day 1 ($P<0.041$) but not on postoperative days 5 to 7 ($P<0.01$). However, none of the patients treated with 2.0 mg/kg h5G1.1-scFv demonstrated new language deficits on postoperative days 5 to 7 compared with 44% of patients who received placebo ($P<0.05$; data not shown). Furthermore, significantly fewer patients treated with 2.0 mg/kg h5G1.1-scFv experienced new postoperative visuospatial reproduction deficits on postoperative days 5 to 7 (11% versus 55% of those who received placebo; $P<0.035$).

Postoperative Blood Loss

Postoperative chest-tube output was collected to assess the effect of h5G1.1-scFv on blood loss. Chest-tube output
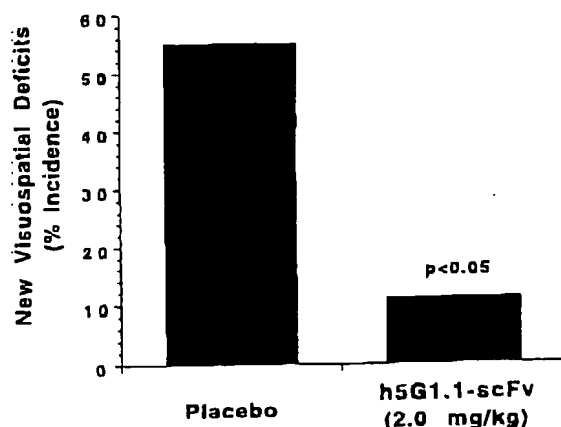

Figure 5. Analysis of cognitive function deficits in CPB patients treated with h5G1.1-scFv. Visuospatial reproductions were determined by serial cognitive function testing in CPB patients before surgery and at hospital discharge. Data shown represent incidences of new visuospatial deficits at hospital discharge for patients who received placebo (n=9) vs 2.0 mg/kg h5G1.1-scFv (n=9).

was significantly less ($P<0.05$) in patients treated with 1.0 or 2.0 mg/kg h5G1.1-scFv (962 ± 133 or 1087 ± 209 mL) than in those given placebo (1474 ± 101 mL). In this small population, there were no differences in transfusions among groups.

Discussion

CPB-induced inflammation is involved in the mediation of postoperative tissue injury and functional impairment of multiple organ systems.[1,4,8] Effective inhibition of this inflammatory response has the potential to significantly reduce clinical morbidity. We report that administration of a potent C5 complement inhibitor (h5G1.1-scFv) to patients undergoing CABG surgery with CPB demonstrated substantial anti-inflammatory activity, as determined by a marked attenuation of complement and leukocyte activation. Administration of h5G1.1-scFv was also associated with significant improvements in several important measures of clinical outcome, including reductions in myocardial injury, new cognitive deficits, and blood loss.

There is substantial evidence that activated components of the complement system play a central role in CPB-associated inflammation and tissue injury. First, elevated levels of activated complement byproducts can be detected in patients undergoing CPB and have been found to correlate with morbidity and mortality.[14,15] Second, the byproducts of C5 complement cleavage, C5a and C5b-9, possess multifaceted proinflammatory properties that contribute to tissue injury in the setting of CPB.[12] C5a is a potent anaphylatoxin, vasoconstrictor,[26] and chemotactic factor for leukocytes.[27] Both C5a and C5b-9 promote leukocyte/endothelial cell interaction via the upregulation of adhesion molecules, such as selectins and the integrin MAC-1 (CD11b/CD18), and alter blood vessel integrity and hemostasis. Both C5a and C5b-9 also amplify inflammatory responses by stimulating cells to release additional inflammatory mediators.[12] Finally, C5b-9 can mediate tissue injury directly because of its lytic properties.[12]

In addition to having intrinsic proinflammatory properties, activated complement byproducts also interact with components of other inflammatory pathways. Thus, contact system proteins (factor XIIa and kallikrein) and products of the coagulation cascade (thrombin and plasmin) have been reported to directly activate complement.[2-4] The products of these other humoral pathways may also amplify complement-mediated activation of leukocytes and platelets.[2-4] Finally, complement proteins can act synergistically with a number of cytokines in promoting inflammatory responses, as exemplified by the C5 dependence of tumor necrosis factor elaboration.[28,29]

There is substantial preclinical evidence to support a critical role for complement in mediating inflammation and tissue injury associated with the CPB-induced inflammatory response. Ex vivo recirculation of whole blood in an extracorporeal closed-loop bypass circuit has been studied extensively as a model to reconstruct the bioincompatibility-induced complement and leukocyte activation that occurs during CPB.[17] The addition of inhibitory anti-C5 antibodies to this circuit completely blocked

the generation of C5a and C5b-9, as well as the upregulation of leukocyte CD11b expression. These studies established that activation products of C5 cleavage, rather than upstream components of the complement cascade (C3a), are the predominant mediators of leukocyte activation in this model.

Studies have further established complement as an important mediator of inflammation and tissue injury secondary to ischemia and reperfusion.[30] It has recently been shown that C5a is responsible for nearly 90% of chemotactic activity in cardiac lymph in the first 4 hours after reperfusion of canine myocardium.[31] We[18] have recently shown in a rodent model of myocardial ischemia and reperfusion that C5a and C5b-9 are key mediators of inflammation and tissue injury in myocardial ischemia and reperfusion. Administration of an anti-C5 mAb before myocardial ischemia and reperfusion dramatically reduced neutrophil infiltration, loss of high-energy phosphate stores, and infarct size.

These preclinical findings provided a firm rationale for the therapeutic trials of the recombinant, single-chain antibody C5 inhibitor in patients undergoing CPB. In the present study, h5G1.1-scFv proved to be a potent inhibitor of systemic complement activation, inhibiting both complement-dependent hemolytic activity and, more importantly, the generation of the proinflammatory activation product C5b-9. Consistent with all preclinical data, this potent complement inhibitory activity was associated with a significant anti-inflammatory effect, as illustrated by the significant inhibition of leukocyte CD11b upregulation achieved in the higher-dose treatment groups. Most importantly, the potent complement inhibitory and anti-inflammatory activities of h5G1.1-scFv were associated with significant reductions in postoperative CK-MB release, new cognitive deficits, and blood loss.

In the present study, the potent inhibitory and anti-inflammatory effects of h5G1.1-scFv were associated with significant reductions in postoperative myocardial injury. The reported incidence of MI after CABG surgery ranges from 1% to 10%.[23] Mechanisms for MI after CABG are likely multifactorial and include preoperative, intraoperative, and postoperative ischemic times, postoperative reperfusion, systemic inflammation, and inadequate revascularization.[23–25] According to postmortem studies,[32,33] 80% to 92% of post-CABG MIs occur without clinical evidence of transmural infarction. Elevated postoperative CK-MB levels are associated with an increasing incidence of postoperative ventricular regional wall motion abnormalities[34] and decreased global left ventricular ejection fraction in the early post-CABG period,[34,35] which can persist for up to 9 months regardless of the presence of Q waves on ECG.[36] There does not appear to be a threshold effect, but rather, it is apparent that the greater the release of CK-MB, the greater the subsequent morbidity, cost, and mortality.[37–40] Hence, it is likely that significant reductions in postoperative myocardial injury might be associated with improved outcomes. The more potent effect of the 2.0 mg/kg dose of h5G1.1-scFv may be related to the longer pharmacodynamic effect, which extends to 12 hours after

CPB, because previous investigators have noted that most postoperative ischemic events occur in the first 10 hours after CPB.[41] Single-chain antibodies have been shown to penetrate tissue more rapidly in vivo than their whole-antibody counterparts, and thus, the myoprotective effect of h5G1.1-scFv may have been related to its capacity for rapid tissue penetration.

Compared with patients who received placebo, those treated with h5G1.1-scFv experienced fewer cognitive deficits. C5 inhibition has similarly been shown to reduce cerebral edema and infarct volume in a rodent model of central nervous system ischemia and reperfusion (S. Rollins, PhD, unpublished data, 1998). The spectrum of central nervous system morbidities after CPB includes cognitive dysfunction, encephalopathy, stroke, and brain death.[20,42] Cognitive dysfunction is the most prevalent and subtle manifestation of central nervous system morbidity, occurring in 30% to 75% of patients after CPB and in up to 40% of patients 2 months after discharge.[43–46] The cause of the cognitive decline is believed to be related to multifocal ischemic and/or hypoxic insults to the brain, leading to neuronal loss.[47–51]

In the present investigation, the cognitive measure used was the MMSE, which assesses global cognitive function. However, despite the constraints of a small sample size and the sensitivity/specificity of the MMSE, the observation that significantly fewer patients treated with the 2 mg/kg dose of h5G1.1-scFv exhibited visuospatial deficits and language deficits on postoperative days 5 to 7 than the placebo group suggests that h5G1.1-scFv may have the potential to ameliorate post-CPB cognitive decline and warrants further investigation.

Blood loss and the subsequent need for transfusion remain a significant complication of CPB. Recent efforts with antifibrinolytic agents have demonstrated reductions in blood loss, but concerns have been raised regarding prothrombotic complications.[52] Reduction of complement activation via heparin coating of oxygenators and tubing has been associated with moderate decreases in blood loss.[53] Furthermore, a correlation has been shown between the degree of complement activation and the amount of postoperative blood loss.[54] Administration of h5G1.1-scFv was associated with a significant reduction in postoperative chest-tube output. Because h5G1.1-scFv does not affect the normal homeostatic regulation of coagulation cascade components, it would not be expected to promote a prothrombotic state.

In summary, we have shown that a humanized, single-chain antibody specific for human C5 is a safe and effective inhibitor of pathological complement activation in patients undergoing CPB. C5 inhibition in patients undergoing CPB produced a pronounced anti-inflammatory effect and was associated with significant reductions in myocardial injury, new cognitive deficits, and blood loss. This provides preliminary evidence that complement inhibition at C5 may reduce clinical morbidity associated with CPB. The clinical findings must be interpreted as preliminary in view of the small number of patients assigned to each treatment group, and they await

confirmation in larger studies. The results of this study may also have implications for the use of the single-chain C5 complement inhibitor in other clinical settings associated with inflammation secondary to tissue ischemia and reperfusion, such as MI or stroke with thrombolytic therapy, and in percutaneous angioplasty procedures.

Acknowledgment

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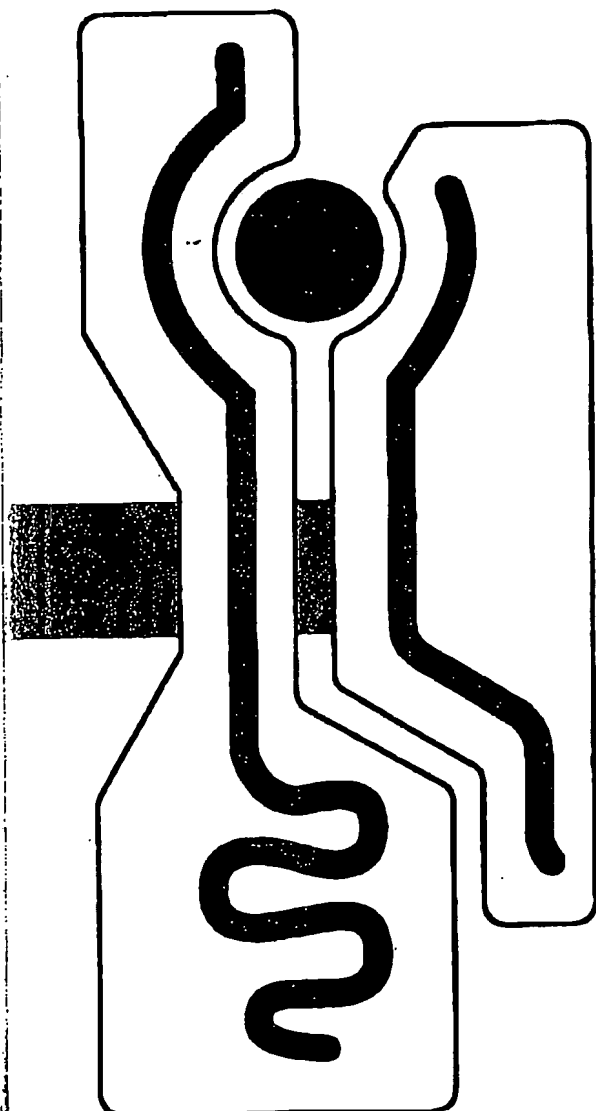
Immunopharmacology

SPECIAL ISSUE

**Abstracts presented at the XVIIIth
International Complement Workshop**

Including 8 Review articles

Salt Lake City, Utah, USA
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195- THERAPEUTIC INTERVENTION IN ACUTE AND CHRONIC DISEASE WITH ANTI-C5 THERAPY.

Scott A. Rollins Ph.D. Alexion Pharmaceuticals Inc.

The complement system is thought to play a critical role in the pathogenesis of multiple disease processes. Although multiple animal models of human disease have been utilized to substantiate the role of complement via therapeutic intervention with complement inhibitors, little is known about the role of complement in human disease. In order to assess the role of complement in acute cardiovascular (cardiopulmonary bypass surgery, myocardial infarction) and chronic autoimmune (rheumatoid arthritis, lupus, membraneous nephritis) diseases, Alexion Pharmaceuticals has developed potent monoclonal antibody-based inhibitors of the human C5 complement component. Human clinical trials have been performed to assess the safety, biological activity and clinical efficacy of C5 inhibition. Preliminary results from Phase I-II trials demonstrate that C5 inhibition in patients undergoing cardiopulmonary bypass is safe and significantly reduces leukocyte activation, myocardial injury, cognitive dysfunction and blood loss. Similarly, Phase I-II studies performed in rheumatoid arthritis patients revealed that C5 inhibitor therapy was safe and significantly reduced C-reactiveprotein levels. Alexion is currently performing large Phase II clinical efficacy trials in several disease settings including: cardiopulmonary bypass surgery, myocardial infarction, rheumatoid arthritis and membraneous nephritis.

196- NOVEL INHIBITORY MONOCLONAL ANTIBODIES TO HUMAN CR2/CD21 LIGAND BINDING DOMAINS

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Complement receptor type 2 (CR2, CD21) is a B lymphocyte cell surface molecule that plays a central role in immune responses to antigen. CR2 is the receptor for the iC3b and C3d fragments of complement component C3 and for the immunoregulatory molecule CD23 (FcERII). Human CR2 is also the B lymphocyte receptor for the Epstein-Barr virus. Several monoclonal antibodies (mAb) react with human CR2, but few bind to the ligand binding SCRs. OKB7, which is no longer commercially available, and mAb FE8 described by Prodinger, et.al., are the only well described mAbs that are able to directly block ligand binding to CR2. We have used fully functional human SCR1,2 protein produced in Pichia pastoris to hyperimmunize CR1/CR2 knockout mice. This approach allowed us to identify four new anti-human CR2 mAbs that bind to structurally important sites in the CR2 SCR1,2 domains. We have analyzed the ability of the mAbs to affect ligand binding to C3d and gp350 in solid phase ELISA and Biacore studies as well as rosetting of hCR2 transfected K562 cells and EBV infection of human peripheral blood cells. Human-mouse intra-cysteine chimeras were used to map sites on CR2 SCR1,2 that affect the binding of these mAbs. Two mAbs (171 and 1048) very efficiently blocked C3d ligand binding and are substantially more effective than OKB7 in a dose-response analysis. Importantly, based on intra-cysteine chimera analysis, they recognize different epitopes than mAb OKB7, suggesting that structure-function data dependent upon use of OKB7 should be re-evaluated. Use of linear peptides to map epitopes suggests these mAbs recognize conformational epitopes on the CR2 structure. Current studies focus on defining these epitopes.

197- AN IGG FC FRAGMENT PEPTIDE BLOCKS INTERACTION OF IGG WITH C1Q

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We have reported that circulating immunoglobulin regulates complement binding to antibody coated targets and have suggested that IVIg acts in part in autoimmune disease by down regulating complement mediated tissue damage. The Fc fragment of IgG is responsible for this profound anti-complementary effect. To localize the active amino acid sequence in the Fc fragment, an IgG1 myeloma protein was purified to homogeneity. Fc fragment peptides were prepared by digestion with insolubilized pepsin at pH 3.5. Active fractions were separated by FPLC superose 12 gel filtration, sephadex peptide and HPLC C-18 chromatography. An active acidic peptide was isolated which markedly inhibits C4 binding to EA in serum. This peptide is located in a highly exposed, hydrophilic portion of the CH3 domain of IgG. Mass spectrometry yielded a mass of 1174 and mass spectrometry microsequencing and traditional direct microsequencing yielded a sequence identical to residues 381 to 390 (W E S N G Q P E N N) of the human IgG1 CH3 domain. (Calculated pI: 3.79.) A synthetic peptide also inhibits C4 binding to antibody coated targets. Control peptides from the CH3 domain of IgG are inactive. ELISA data suggests that C1q interacts with peptide coupled to BSA. The BSA-peptide prevents C1q binding to target cells and blocks the ability of C1q to reconstitute the lytic activity of C1q depleted serum. BSA or control peptide-BSA are inactive. The evidence suggests that this region is a new C1q binding site. The exposed, hydrophilic peptide location on the CH3 domain of the IgG heavy chain suggests that this peptide plays a physiologic role in immune regulation.

198- REGULATION OF THE ALTERNATIVE PATHWAY ON INJURED CELLS BY FACTOR H, FHL-1 AND C-REACTIVE PROTEIN

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Factor H (FH) and its truncate form FHL-1 are the major fluid phase regulators of the alternative complement pathway. They bind to C3b and have one or more binding sites for cell surface glycosaminoglycans and for C-reactive protein (CRP), the major acute phase protein. To evaluate the physiological relevance of the latter interactions we analyzed FH and CRP binding to the surface of ischemic or injured cells.

Endothelial or lymphoma cells were rendered ischemic by growing them under anaerobic conditions. Alternatively, cell injury was induced by treatment with serum and a complement-activating and CD59-blocking antibody (YTH53.1). Ischemic or complement-attacked cells were incubated with ^{125}I-labeled CRP or FH in the presence or absence of unlabeled CRP.

Following ischemic injury or complement attack the binding of ^{125}I-CRP to cells increased and the binding of ^{125}I-FH decreased. However, in the presence of CRP, the binding of FH to injured cells increased up to 2-fold. The binding of ^{125}I-FHL-1 to injured cells also increased when the cells were pretreated with CRP.

These results indicate that CRP may target FH activity to injured cells or tissues. FH and FHL-1 promote inactivation of C3b to iC3b, thus generating a ligand for macrophages. This, together with the recently identified CRP-FcγRII interaction may directly promote phagocytosis of injured cells.

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ABSTRACT SUPPLEMENT

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